Thermal Stability of Pellets over wide range of temperature

CT Amplification after 10 minutes at Each Temperature (10 μL HPA)

FIG. 1

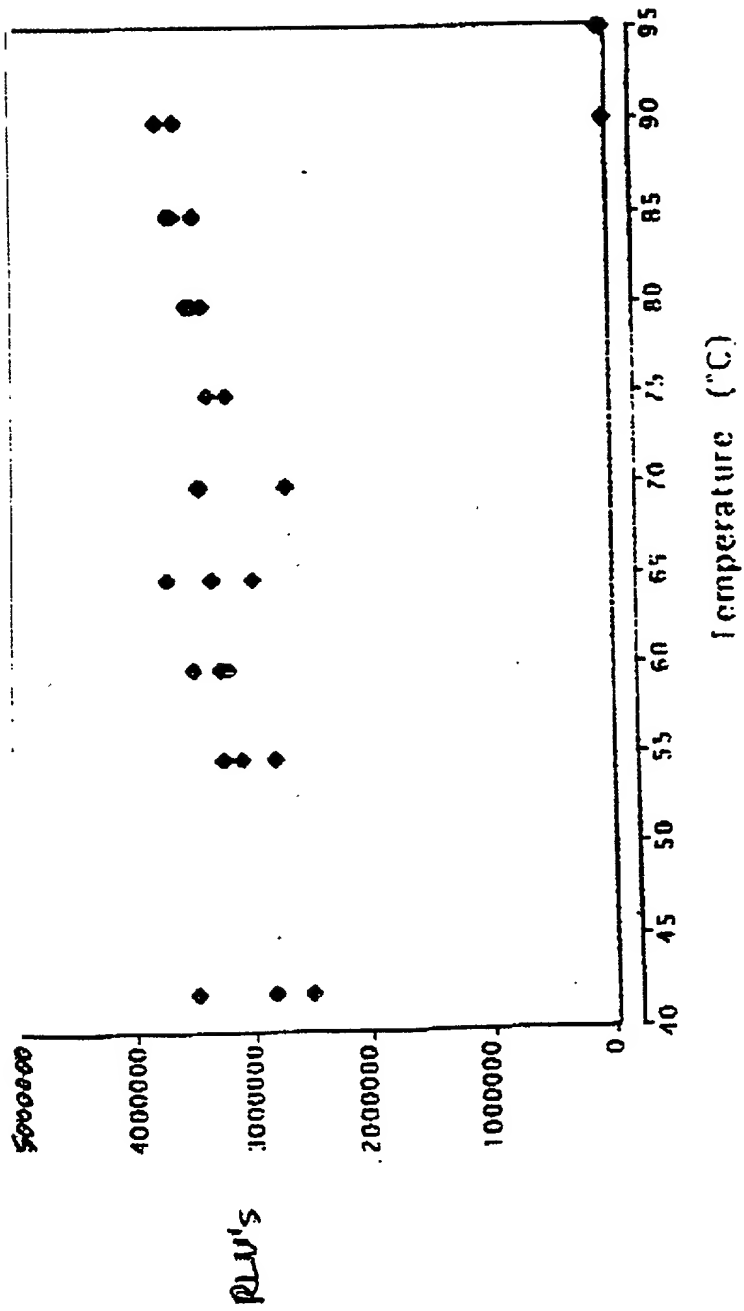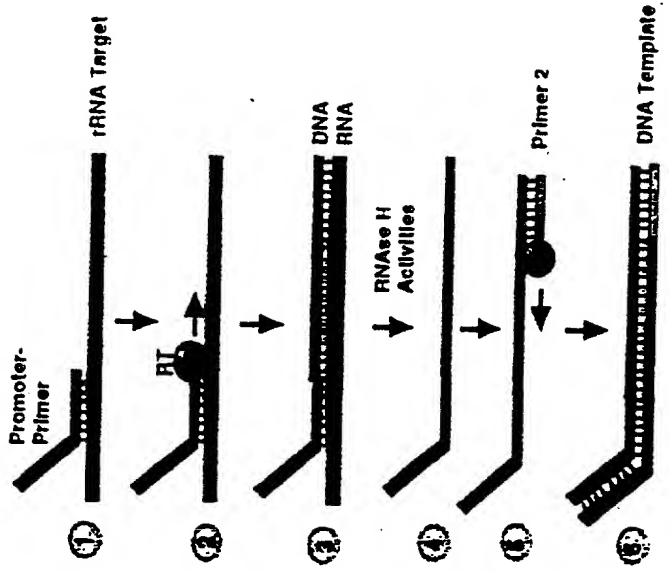bioMérieux   Rockland R&D

Transcription Mediated Amplification

FIG. 2

bioMérieux

Rockland R&D

FIG. 3A

targets / ampmix + enzymes → 42 °C

FIG. 3B

Sample   Sample   OR

Dried Amplification Reagent

Dried Enzyme Pellet
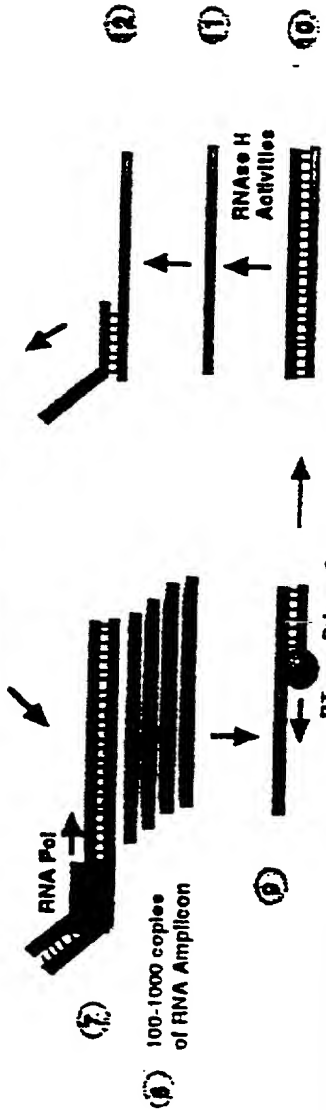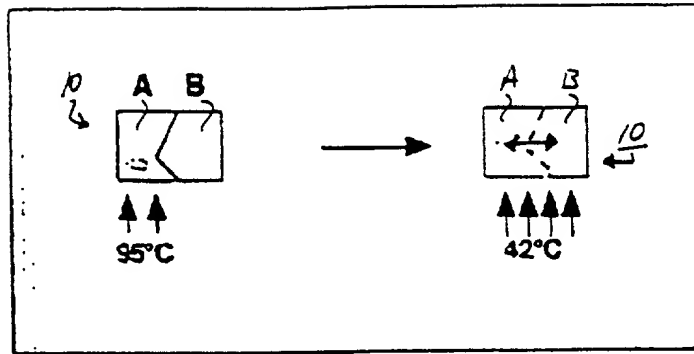
FIG. 3C
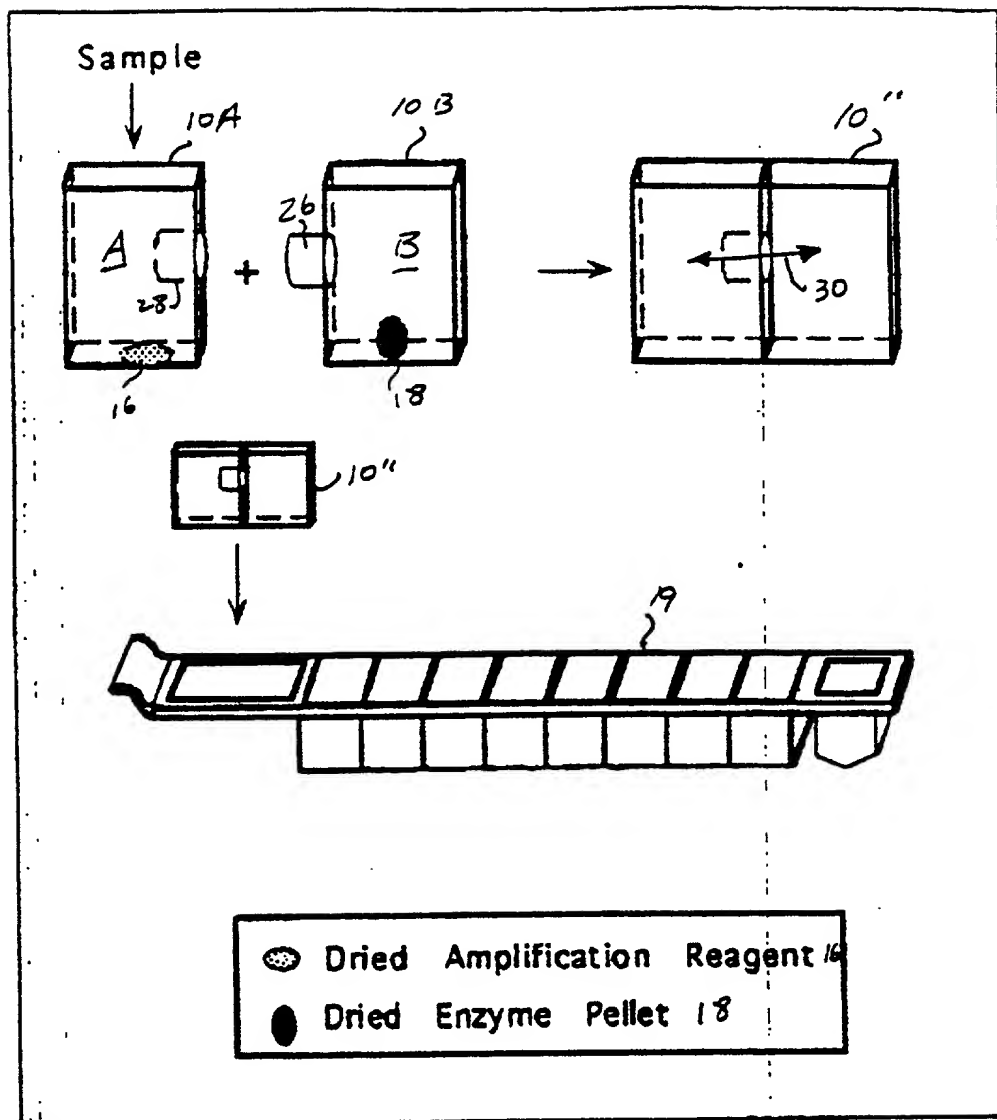
FIG. 4

FIG 5
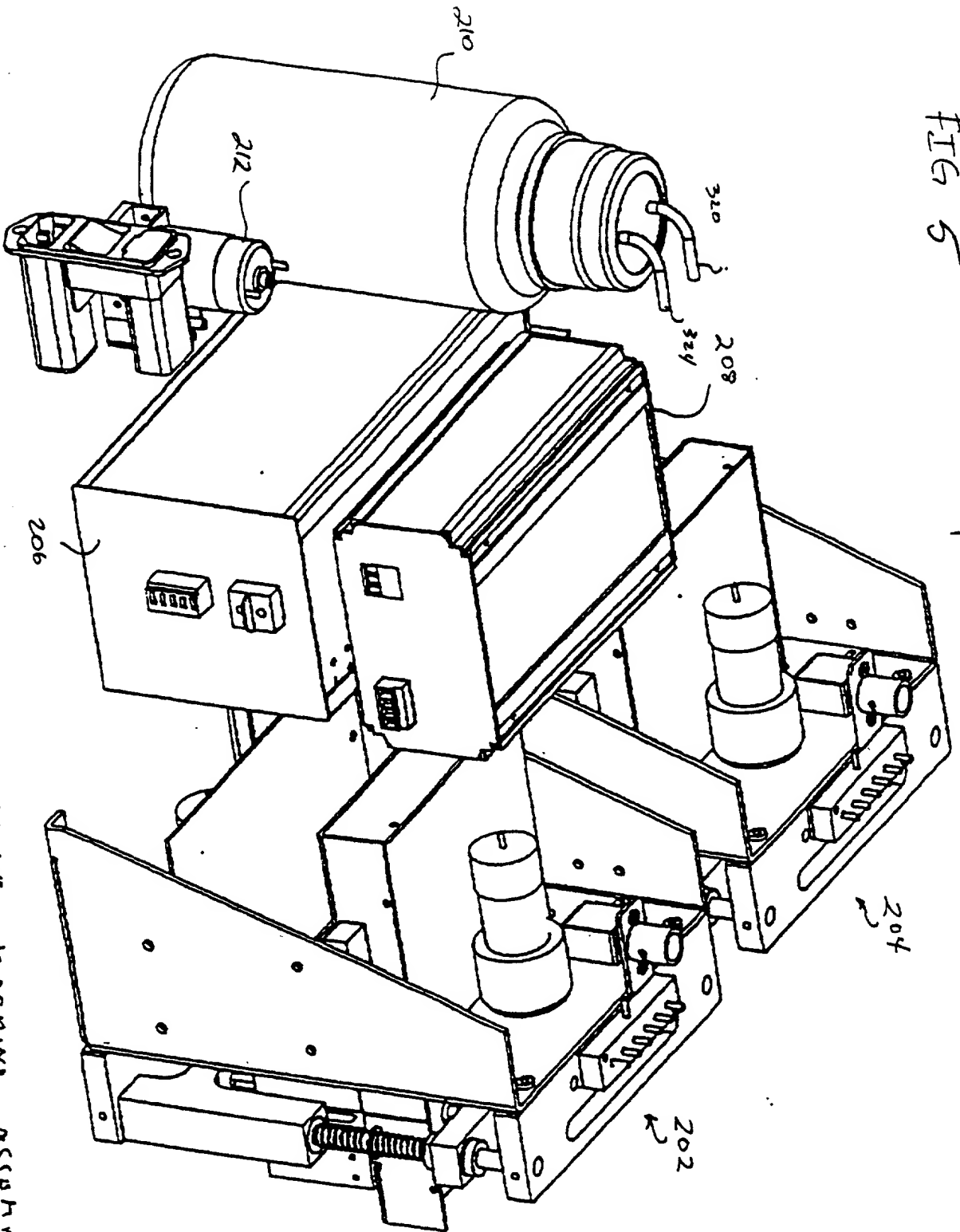
WHOLE MACHINE ASSEMBLY
200

210

212

320

324

208

206

204

202

PINCH 224
VALVE

202

VACUUM
PROBES
SLIDE
MOTOR 222

226

TRAY CONTROLLER BOARD 229

230

234

228

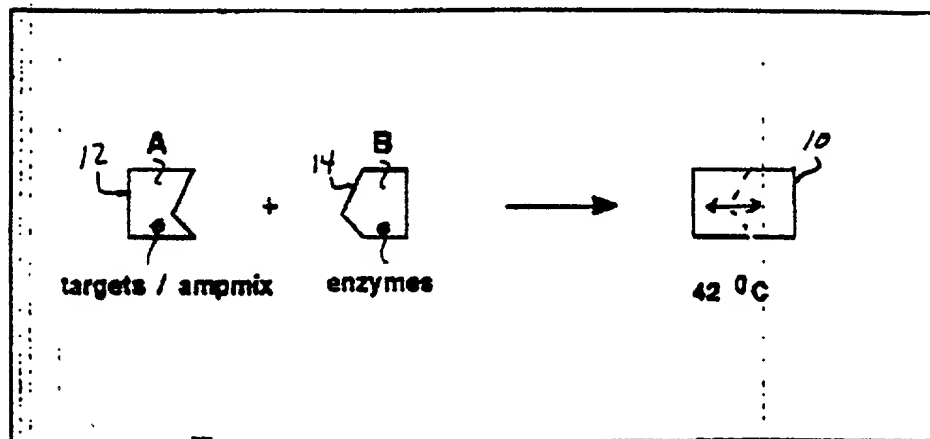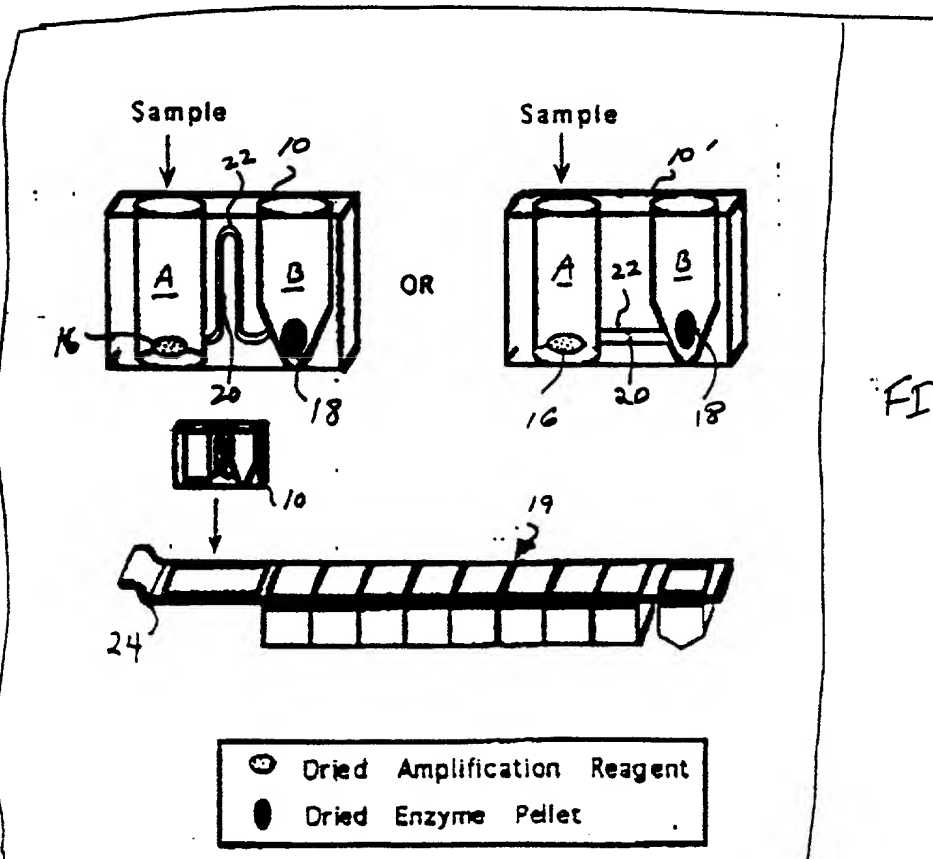TRAY THERMAL INSULATION 220

FIG. 6

FIG. 7

FIG. 8

246

95°C
PELTIER
HEATSINK
250

220

252

242

242

252

BOTTOM
SIDE

UNCOVR
PROBE
GASKET
260

244

4

260

256B

256A

220

220

250

258

256

254

252

240

FIG. 9

FIG. 10

19

241

STRIP 3

A

254

256

250
(95°C)

240

(42°C)
242

252
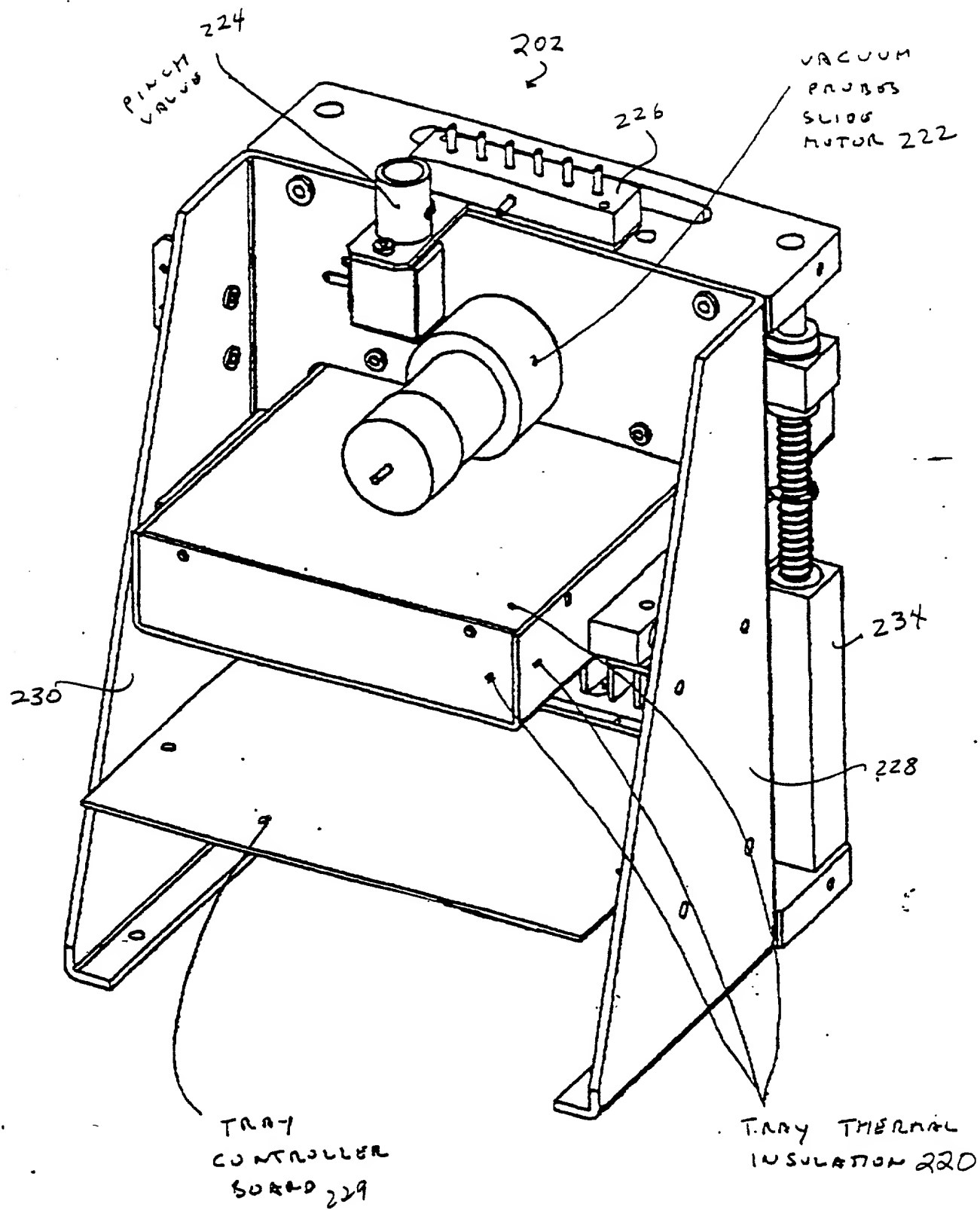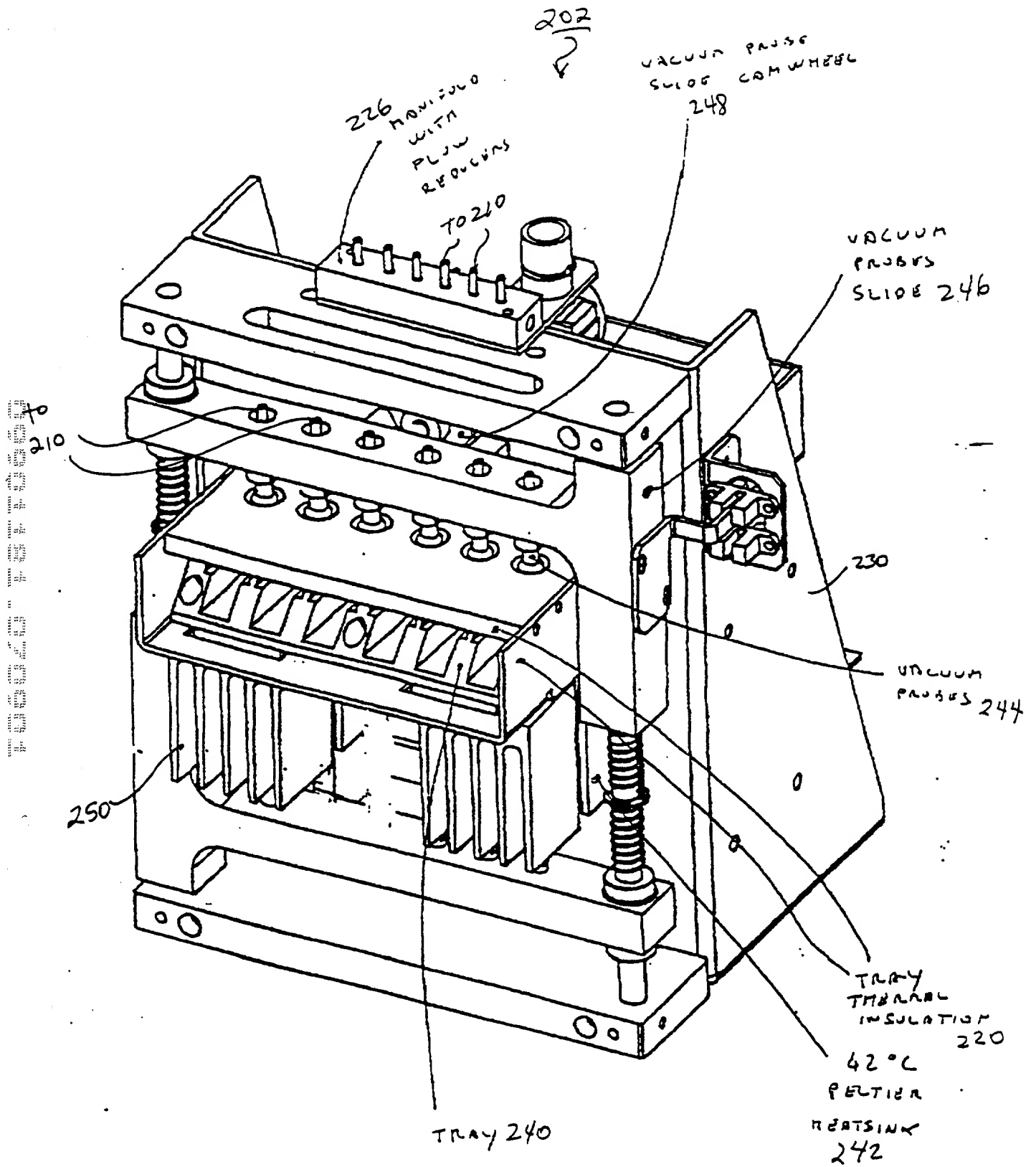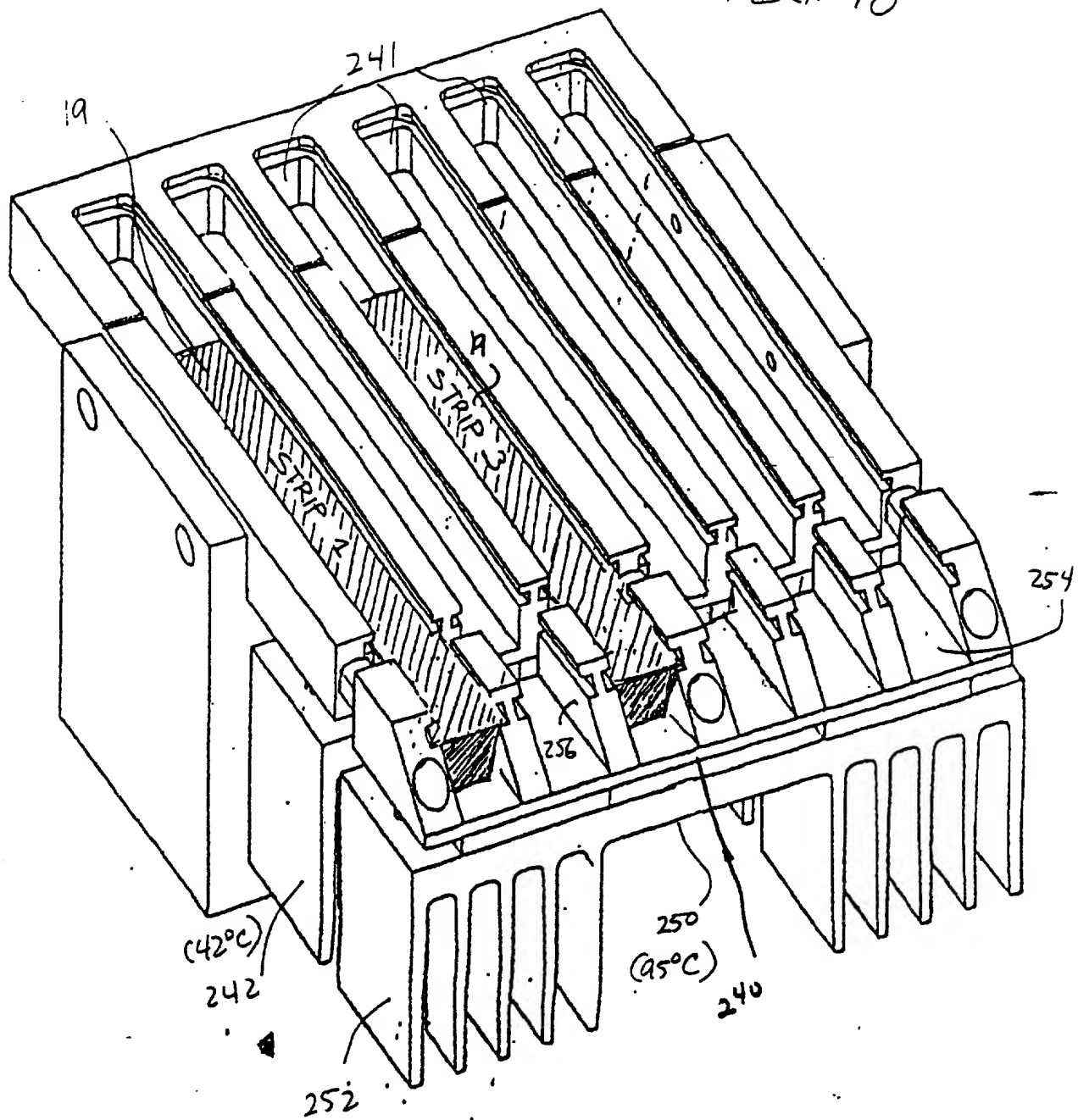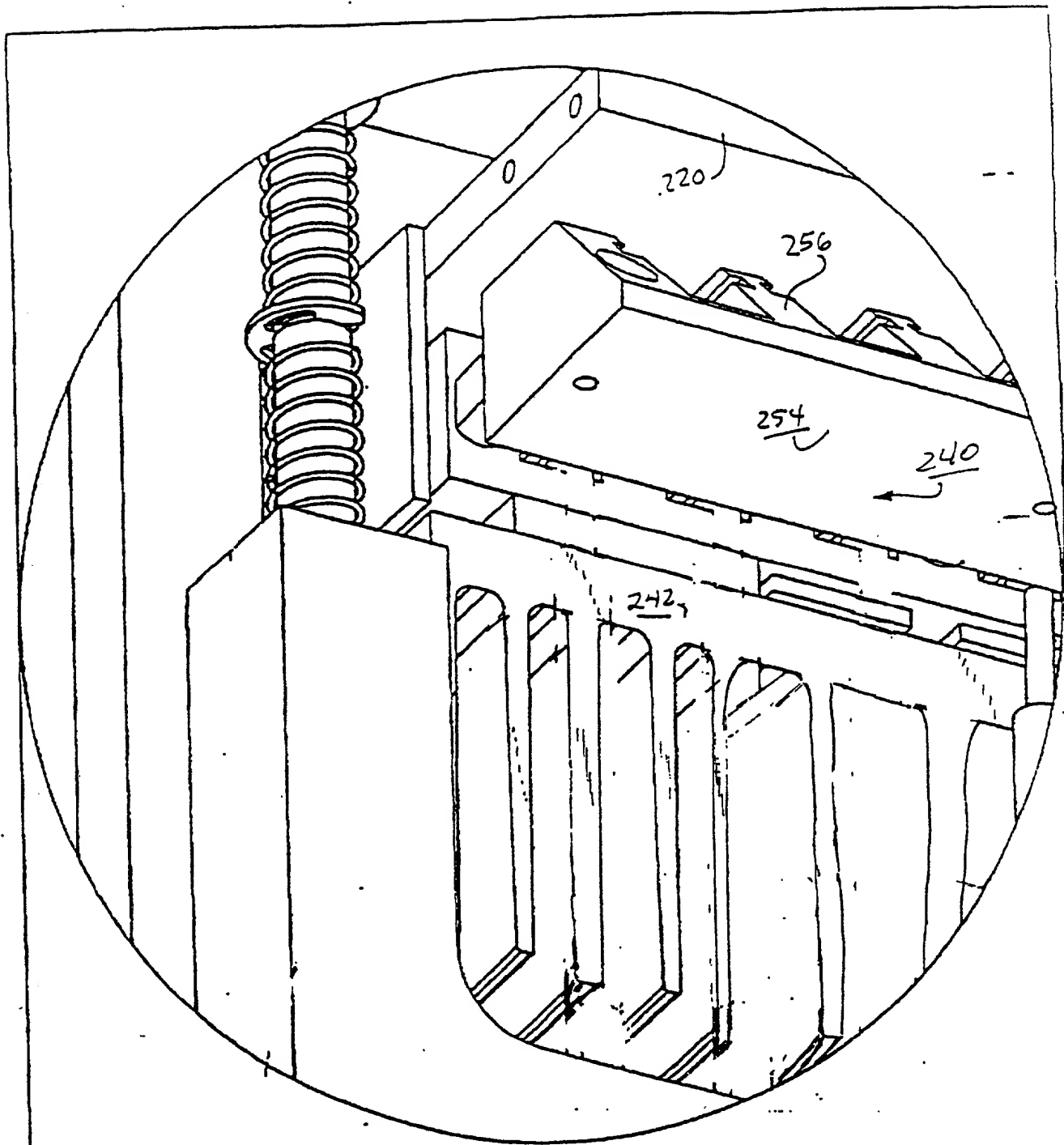
FIG. 11

AMPLIFICATION STATION (BLOCKS DIAGRAM)

FIG 12

FIG. 13

VACUUM SYSTEM DIAGRAM

DIFFERENTIAL PRESSURE TRASDUCER (IRS 286-686)

VACUUM PUMP PSF ASSEO20790

PINCH SOLENOID VALVE (SIRAI S.104.03 EG3 24VC.C.)

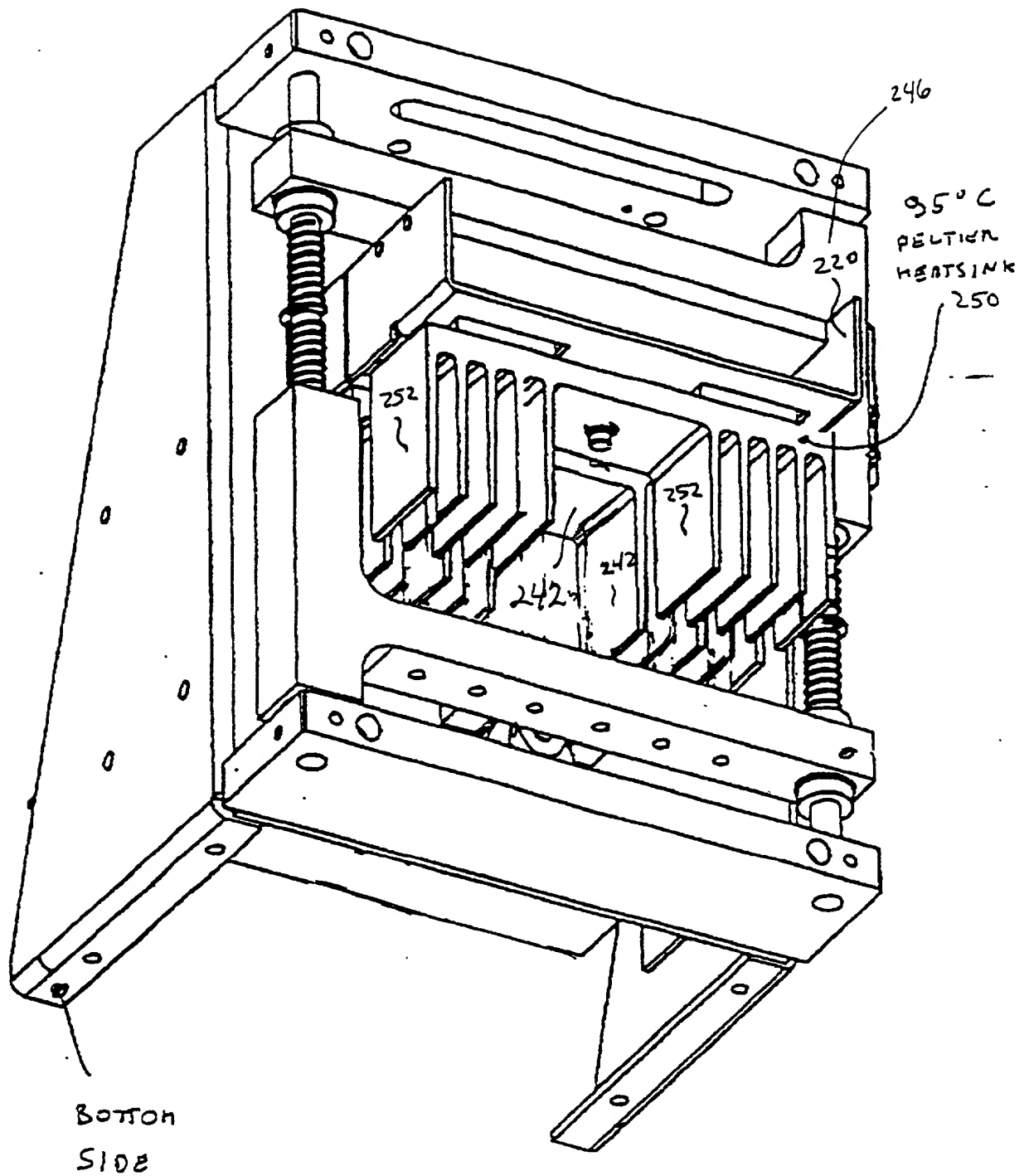MANIFOLDS WITH FLOW REDUCER

TO TRAY 2 (module 204)

TO TRAY 1 (module 202)

PLASTIC TANK (1 LITRE)

fluid transfer (x minutes after cooling)

Temperature (C)

T_1

T_3

T_2

402

404

400

Temp. 2, time 2

Temp. 3
time 3

* ramp up/down time: maximum 1 minute

time (min)

406

408

FIG. 14

1.

2.

Hybridize and Capture

3.

4-MUP

FLUOR

4.

NaOH

5. a. Empty cuvette
of used MUP

b. Wash cuvette

c. Transfer fresh
4-MUP to cuvette

6.

New 4-MUP

NO FLUOR

7.

New 4-MUP

FLUOR

Fig 15

SPR PRODUCTION WITH DISTINCT CAPTURE ZONES

1.   2.   3.   4.

470 μl

90 μl

Zone 2
(2.0 cm^2)

Zone 1
(1.8 cm^2)

Conjugate of AMVE copolymer
and specific capture probe

Fig. 16

MULTIPLEX STRIP CONFIGURATION

Fig. 17

Test of Multiplex VIDAS Protocol

FIG-18

FIG.
19 A. Dose Response Multiplex Detection of CT and NG
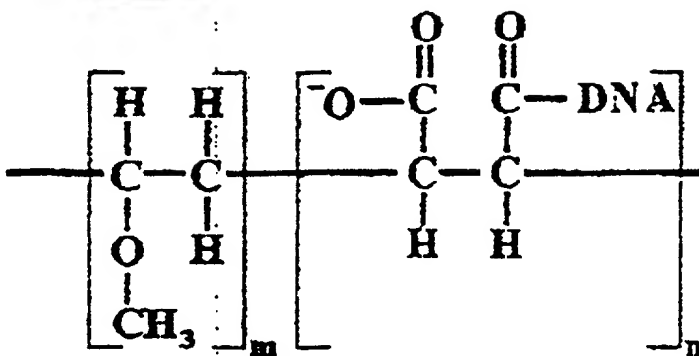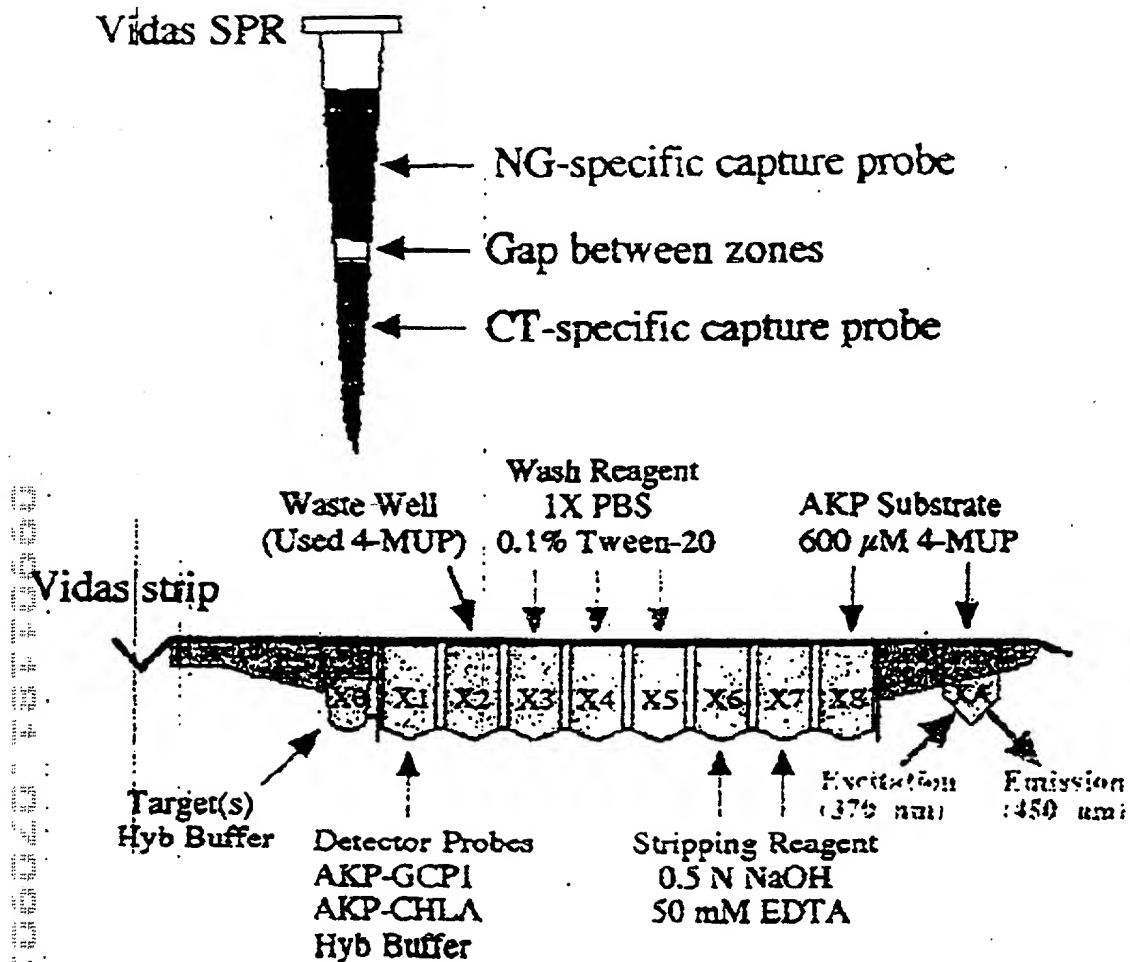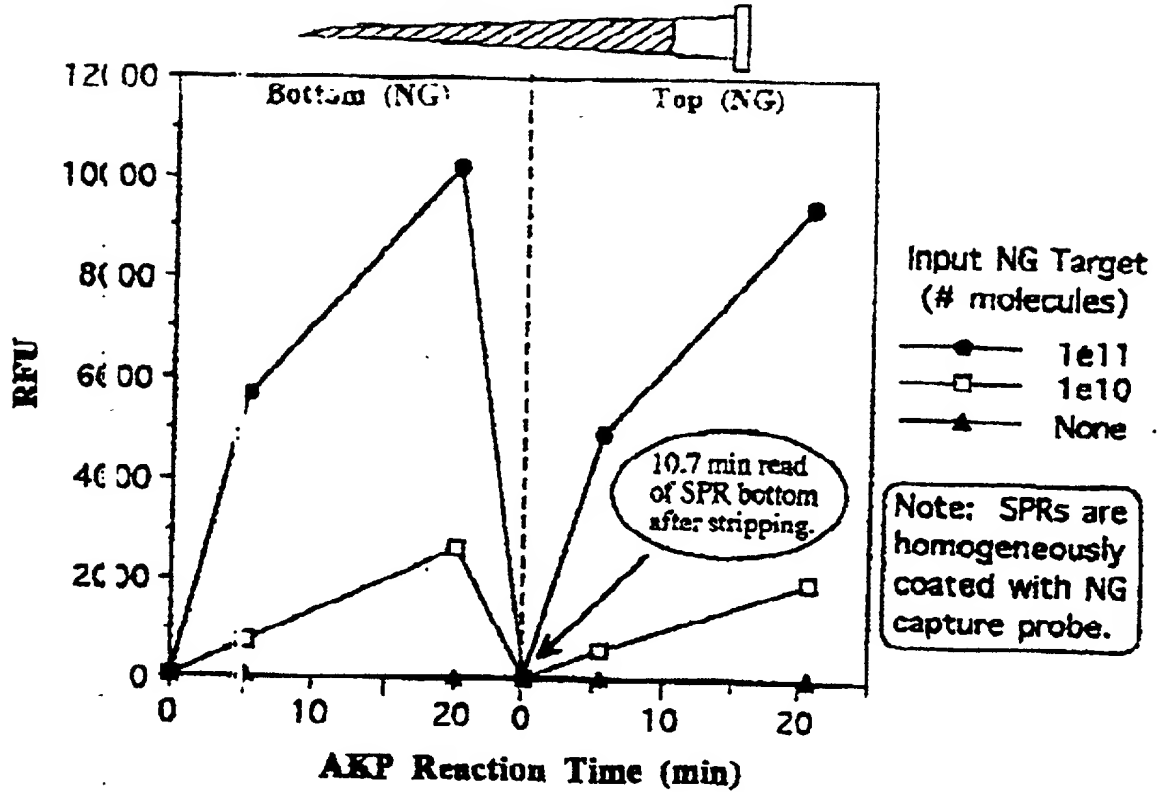

FIG.
19 B.

Fig 19

Fig 20A

Fig. 20B

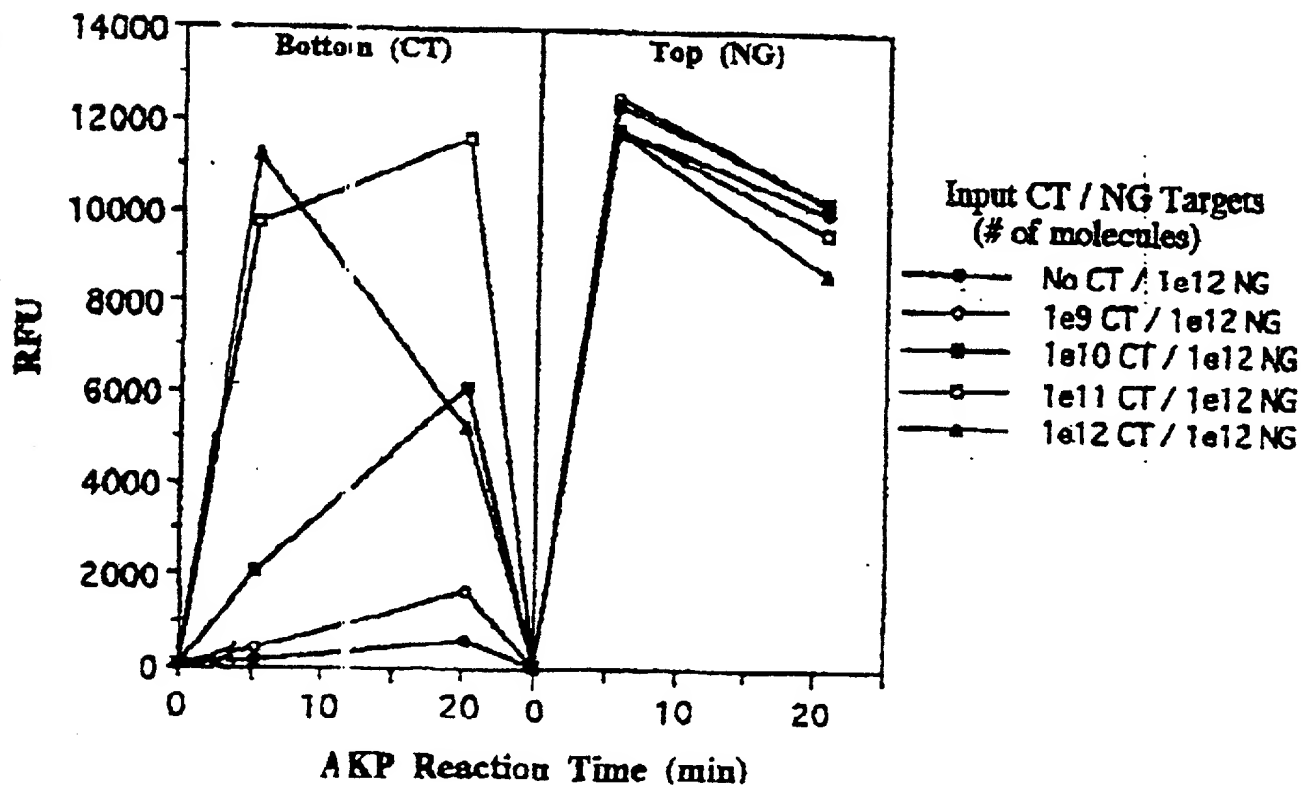cut-off 30000 RLU

cut-off =924 RFU

Amplification & detection on VIDAS

12-7-95
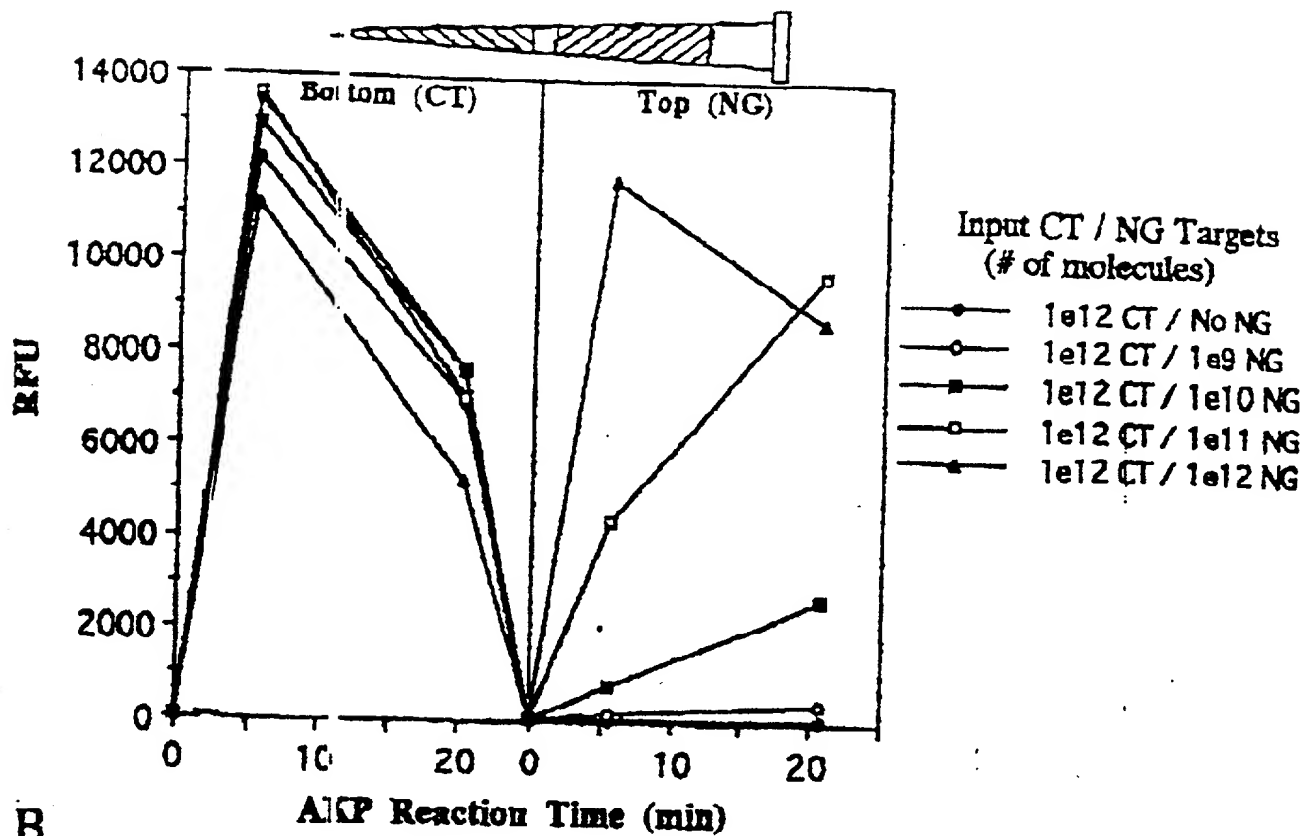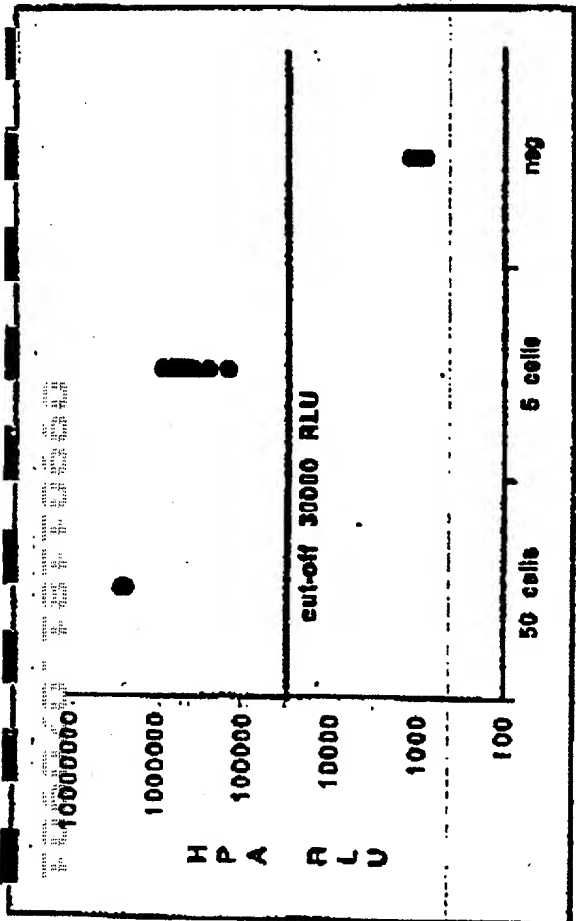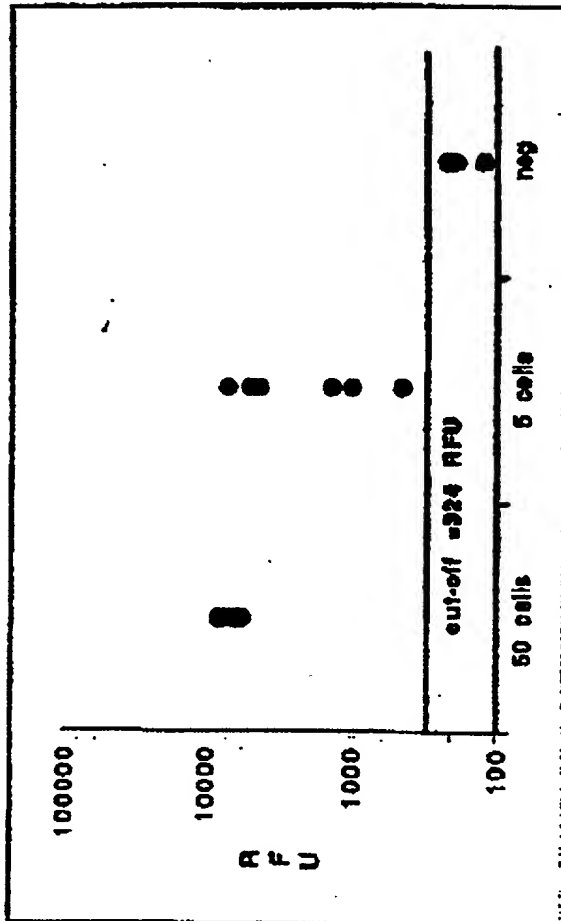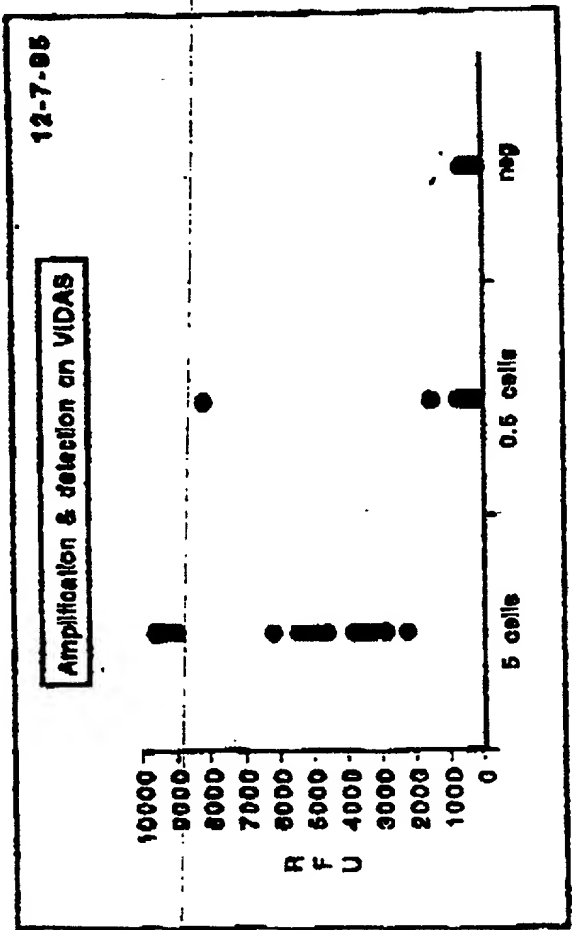
* sample heated off-line in the presence of ampmix, enzyme transfer by VIDAS

Fig. 21

bioMérieux   Rockland R&D

Binary: VIDAS detection

Binary: amplification and detection
on 44°C VIDAS

Legend:
- pellet 3 fg
- liquid 3 fg
- pellet 0 fg
- liquid 0 fg

Axes:
- Y-axis: RFVs (0, 2000, 4000, 6000, 8000, 10000, 12000)
- X-axis: Read Time (0, 10, 20, 30, 40)

bioMérieux   Rockland R&D

FIG. 22

FIG. 23

FIG. 24

Random Internal Control 1

5'-gggAgCgAATgTTAgggCACACTCATgggTgAgCAAgTCTTTCTgTAAgggCTgATgTCAggCgTATTgACAAgCATgACgACCAgA-3'
3'-cccTCgCTTACAATCCCgTgTgAgTgAgTACCCACTCgTTCAgAAAgACATTCCCgACTACAgTCCgCATAACTgTTCgTACTgCTggTCT-5'

RAN16 primer: 5'-AgCgAATgTTAgggCACACTC-3'   5'-TAAgggCTgATgTCAggCgTA-3'   RAN21 AMVE-probe

TARGET: 5'-gggAgCgAATgTTAgggCACACTCATgggTgAgCAAgTCTTTCTgTAAgggCTgATgTCAggCgTATTgACAAgCATgACgACCAgA-3'

RAN33 AKP-probe: 5'-ATgggTgAgCAAgTCTTTCTg-3'   3'-AACTgTTCgTACTgCTggTCT

AgAgggATATCACTCAgCATAATTTAA-5'
(T7 promoter / RAN19 primer)

(T3 promoter)

5'-GCAATTAACCCTCACTAAAGGGAgCgAATgTTAgggCACACTCATgggTgAgCAAgTC-3'
3'-gTACCCACTCgTTCAgAAAgACATTCCCgACTACAgTCCgCATAACTgTTCgTACTgCTggTCT-5'

OLIGOS TO ORDER:

RAN16 TMA primer: 5'-AgC gAA TgT TAg ggC ACA CTC-3'

RAN21 AMVE-probe: 5'-aminolink-TAA ggg CTg ATg TCA ggC gTA-3'

RAN33 AKP-probe: 5'-aminolink-ATg ggT gAg CAA gTC TTT CTg-3'

T7/RAN19 TMA primer: 5'-AAT TTA ATA CgA CTC ACT ATA ggg AgA TCT ggT CgT CAT gCT TgT CAA-3'

RIC1 Detection oligo: 5'-CAA TAC gCC TgA CAT CAg CCC TTA CAg AAA gAC TTg CTC ACC CAT gAg-3'

RIC1 top oligo: 5'-GCA ATT AAC CCT CAC TAA AGG GAg CgA ATg TTA ggg CAC ACT CAT ggg TgA gCA AgT C-3'

RIC1 bottom oligo: 5'-TCT ggT CgT CAT gCT TgT CAA TAC gCC TgA CAT CAg CCC TTA CAg AAA gAC TTg CTC ACC CAT g-3'

RIC 1

FIG. 25

RAN 33 (AKP)

RAN 16

RAN 21 (AmVE)

RAN 19
(COMPLEMENT)

FIG. 26
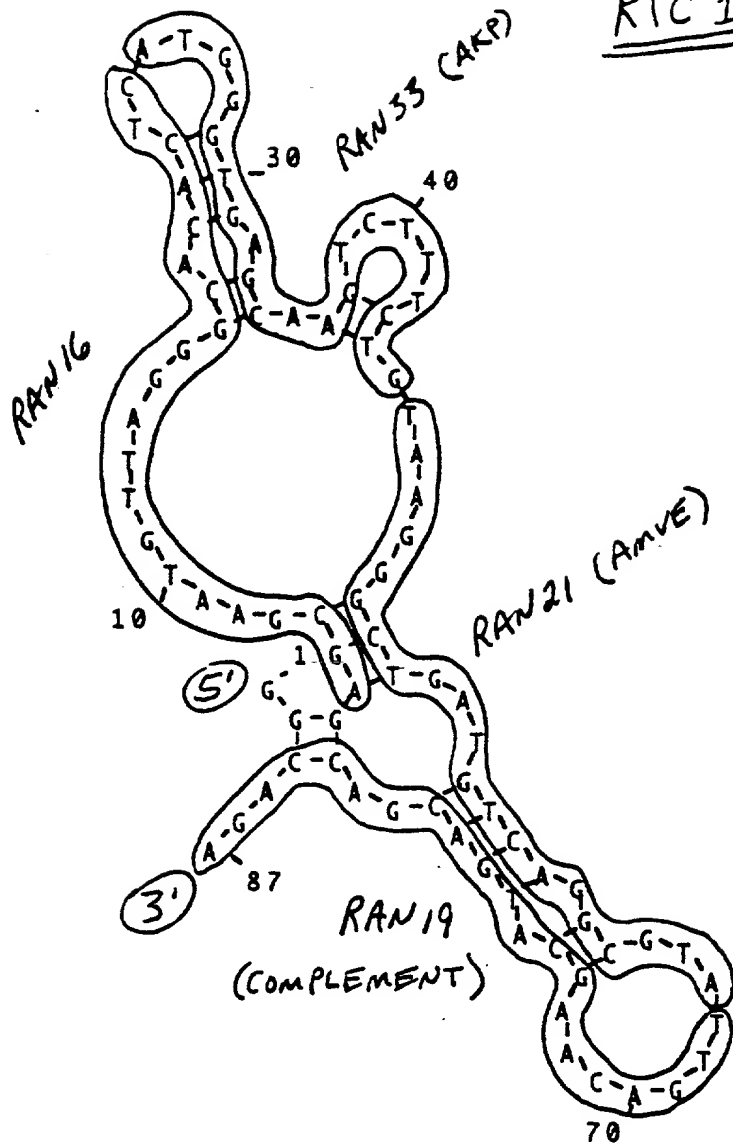
Random Internal Control 2

5'-CAgTAgAggTAggggCTgCTAggAgTATAACAgAAgCCAgTgTACggAACgACTCAgCACgCgAATACTTTgCTACCAgACCTAgAggAgTgCgT-3'

3'-gTCATCTCCATCCCgACgATCCTCATATTgTCTTCggTCACATgCCTTgCTgAgTCgTgCCgCTTATgAAACgATggTCTggATCTCCTCACgCA-5'

RAN51 TMA primer 5'-CAgTAgAggTAggggCTgCTAggAgT-3'

5'-ACgACTCAgCACgCgAATAC-3' RAN32 AKP-probe

TARGET --------> 5'-CAgTAgAggTAggggCTgCTAggAgTATAACAgAAgCCAgTgTACggAACgACTCAgCACgCgAATACTTTgCTACCAgACCTAgAggAgTgCgT-3'

RAN27 AMVB-probe 5'-TAACAgAAgCCAgTgTACggA-3'

3'-ACgATggTCTggATCTCCTCACgCA
AgAgggGATATCACTCAgCATAATTTAA-5'
(T7 promoter / RAN39 primer)

(T3 promoter)
5'-GCAATTAACCCTCACTAAAGGGCAgTAgAggTAggggCTgCTAggAgTATAACAgAAgCCAgTgTAC-3'
                                                     3'-gTCTTCggTCACATgCCTTgCTgAgTCgTgCCgCTTATgAAACgATggTCTggATCTCCTCACgCA-

OLIGOS TO ORDER:

RAN51 TMA primer: 5'-CAg TAg Agg TAg ggg CTg CTA ggA gT-3'

RAN27 AMVB-probe: 5'-aminolink-TAA CAg AAg CCA AAg CCA gTg TAC ggA-3'

RAN32 AKP-probe: 5'-aminolink-ACg ACT CAg CAC ggC gAA TAC-3'

T7 / RAN39 primer: 5'-AAT TTA ATA CgA CTC ACT ATA ggg AgA ACg CAC TCC TCT Agg TCT ggT AgC A-3'

RIC2 Detection oligo: 5'-AAg TAT TCg CCg TgC TgA gTC gTT CCg TAC ACT ggC TTC TgT TAT AC-3'

iC2 Top oligo: 5'-GCA ATT AAC CCT CAC TAA AGG GCA gTA gAg gTA ggg gCT Agg AgT ATA ACA gAA gCC AgT gTA C-3'

RIC2 Bottom oligo: 5'-ACg CAC TCC TCT Agg TCT ggT AgC AAA gTA TTC gCC gTg CTg AgT CgT TCC gTA CAC Tgg CTT CTg-3'

RIC 2

FIG. 27

Detection of RIC1 DNA Oligo Targets

Note: This was run
on VIDAS #1019 which
has a poor extended
range (~8000 RFU).

● ——— 1e10 RIC1
□ ——— 1e11 RIC1

Reaction Time (minutes)

RFU

FIG. 28

AMPLIFICATION OF PURIFIED RIC1 RNA
Detection by ZULU protocol
B. Kluttz data

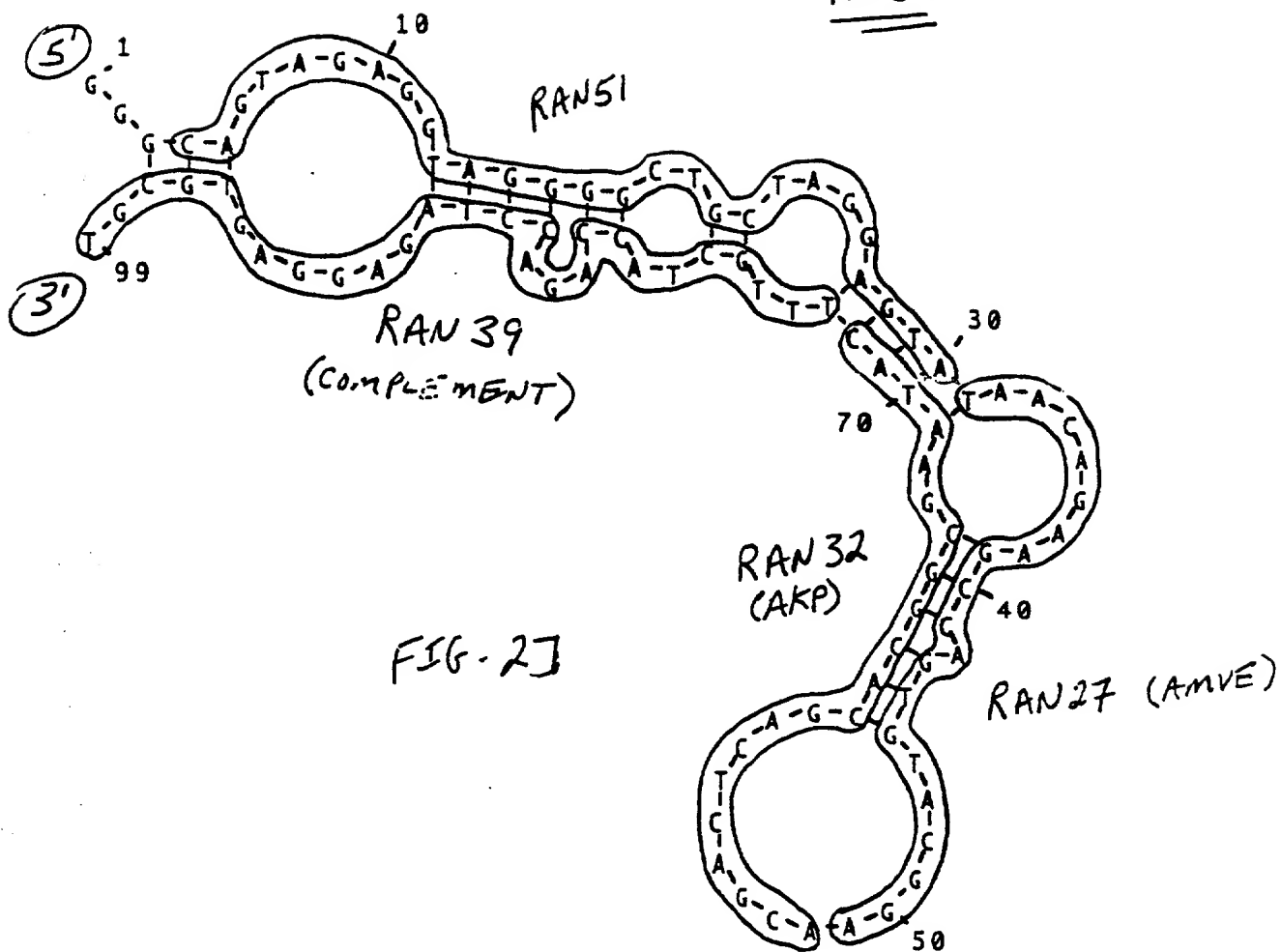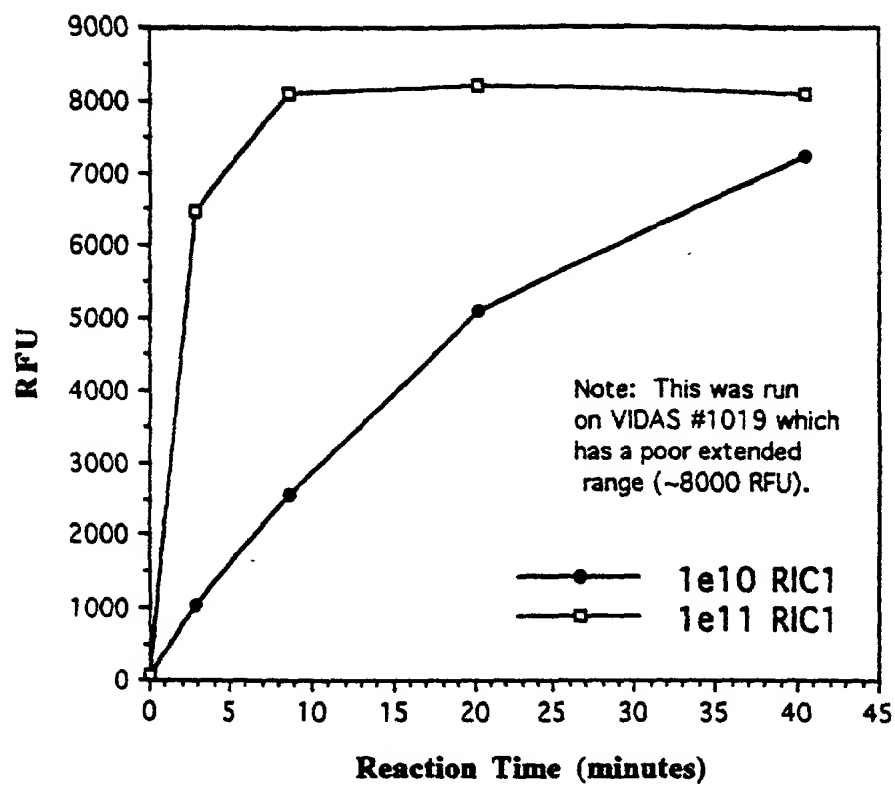| Position	RIC1 RNA*	CT RNA	AKP Type & SPR Type	0 min	1.8 min	5.4 min	14.6 min	40.0 min
C1	none	none	RIC1	56	56	58	61	70
C2	"	none	RIC1	57	55	57	59	66
C3	0.1	none	RIC1	56	55	57	61	70
C4	"	none	RIC1	57	56	57	61	68
C5	1	none	RIC1	56	55	59	65	81
C6	"	none	RIC1	56	55	57	62	74
D1	10	none	RIC1	55	78	114	202	414
D2	"	none	RIC1	56	56	59	66	82
D3	100	none	RIC1	56	55	58	62	73
D4	"	none	RIC1	57	57	61	70	94
D5	1000	none	RIC1	56	58	81	119	227
D6	"	none	RIC1	56	57	70	102	184
E1	10000	none	RIC1	56	93	209	414	948
E2	"	none	RIC1	56	105	246	497	1155
E3	100000	none	RIC1	56	395	1474	3029	6510
E4	"	none	RIC1	56	596	1981	4309	7830
E5	1000000	none	RIC1	56	985	3597	7371	10840
E6	"	none	RIC1	55	1062	3617	7464	10839

Amplification performed with CT reagents, spiked with RIC1 primers (2.5 pmol RAN 16 and 5 pmol T7/RAN 19)

Each sample is an independent amplification.

RIC1 SPRs coated at 0.5 ng/µl instead of the "normal" 1.0 ng/µl level.

FIG. 29